Diseases Caused by Oomycetes

First Report of *Phytophthora pini* Causing Foliage Blight and Shoot Dieback of *Rhododendron pulchrum* in China

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During a 2019 to 2020 survey for plant pathogenic oomycetes in Nanjing, China, severe foliage blight and dieback were observed on approximately 20 *Rhododendron pulchrum* plants at three public parks and gardens. Approximately 25% of leaves and shoots were affected. Symptoms included brown to black lesions on leaves and stems, dieback of shoot tips, and wilting. Diseased tissues were collected from a 5-year-old shrub with typical disease symptoms at Xuanwuhu Park. They were cut into 10 × 10-mm squares, immersed in 70% ethanol for 30 s, and placed onto fresh clarified V8 juice agar (cV8A) containing pimarin, ampicillin, rifampicin, and pentachloronitrobenzene. *Phytophthora*-like hyphae were transferred to new cV8A plates daily. A total of five isolates were obtained after 5 days of incubation at 25°C. After approximately 20 days, all isolates were identical in morphological traits including semipapillate sporangia and gametangia (homothallic). Thirty sporangia of representative isolate Ppi were randomly selected and examined. They were mostly ovoid and sometimes obpyriform, averaging $41.0 \pm 3.9 \times 24.8 \pm 3.2 \mu\text{m}$. Antheridia of 30 randomly selected gametangia were parangynous, averaging $16.7 \pm 0.7 \times 12.4 \pm 1.5 \mu\text{m}$. Average diameters of oogonia and plerotic oospores were 29.2 ± 0.3 and $26.4 \pm 1.6 \mu\text{m}$, respectively. Chlamydospores were not observed. The above morphological traits suggested the causal agent

belonging to the *Phytophthora citricola* complex (Hong et al. 2011). Isolate Ppi was subjected to sequencing of the rDNA internal transcribed spacer (ITS) region and the *ras*-related GTP-binding protein 1 (*Ypt1*) gene. ITS sequence of Ppi (GenBank no. MT672594) has 100% identity to that of *P. pini* (MG865565). It has a 3-nt difference from the ITS sequences of *P. acerina* (MG518642) and *P. citricola* (MG865475) and a 4-nt difference from that of *P. plurivora* (FJ665225). *Ypt1* sequence of Ppi (MT680000) has 100% identity to that of *P. pini* (MK058416). Pathogenicity of Ppi on *R. pulchrum* was tested using both detached-leaf and whole-plant assays. In the former assay, each of six asymptomatic leaves was symmetrically wounded on both sides using a sterile inoculation needle. A 5 × 5-mm Ppi-colonized cV8A plug was placed on each wound of five leaves. Sterile agar plugs were used for a control leaf. All six leaves were placed on a wet filter paper in a closed container at 25°C. This assay was repeated twice. On the fifth day, all inoculated leaves had necrotic tissues around the wounds, whereas the control leaves remained asymptomatic. In the whole-plant assay, 20-inch-tall plants were used. Five attached leaves and the twig base of each plant were wounded. A control plant was inoculated in the same manner as above, and sterile agar plugs were used. Each plant was covered with a plastic bag and maintained at 25°C. Wet cotton balls were placed in the bags to maintain humidity. After 2 days, the bag containing cotton balls was removed. This assay was repeated three times. After 2 weeks, all three inoculated plants in the three replicated trials had severe foliage blight and dieback, whereas control plants remained healthy. *Phytophthora* isolates recovered from artificially inoculated tissues were identical to isolate Ppi in morphological characters. *Rhododendron* diseases caused by *P. pini* were reported in the United States (Knaus et al. 2015; Loyd et al. 2014) and Finland (Rytönen et al. 2012). This is the first report of *P. pini* causing foliage blight and dieback on *R. pulchrum*, an important nursery and landscape plant in China. Additional surveys are ongoing to determine the distribution of this pathogen in Nanjing. Management programs are under development to contain the spread of *P. pini* and treat diseased plants.

References:

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